Fabrication of Calcium Hydroxide/Manganese Oxide Nanocomposite as a Novel Antibacterial Agent

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Abstract: The emergence of microbial resistance in bacterial pathogens to common antimicrobial agents is a significant challenge in their use. The purpose of this research is to investigate the antibacterial properties of a nanocomposite consisting of calcium hydroxide and manganese oxide against the oral pathogen *Enterococcus fecalis.* To determine the antibacterial effect of nanocomposites on *E. faecalis,* 9 experiments were designed using the Taguchi method. In the experimental investigations, three factors of calcium hydroxide, manganese oxide nanoparticles, and their stirring time were investigated. These agents had three different levels and finally led to the identification of the optimal ratio that showed the greatest antibacterial effect. The nanocomposites made using test conditions 6 (calcium hydroxide 150 mg/mL, manganese oxide 9 mg/mL, and stirring time 60 minutes) showed the greatest effect in growth inhibition (0.29 CFU/mL). The properties of the synthesized nanocomposite and its components were evaluated through material characterization techniques. The structural properties and chemical composition of this nanocomposite were found to be favorable based on its characteristics. As a result, the nanocomposite consisting of calcium hydroxide and manganese oxide has beneficial antibacterial properties that make it suitable for increasing performance in various dental fields.

Keywords: Nanocomposite; Calcium hydroxide; Manganese oxide; Antimicrobial Resistance; Taguchi method.

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Novel nanocomposite as antibacterial agent

1. INTRODUCTION

In root treatments, the main goal is to prevent or treat apical periodontitis. Complete cleaning of root canals is difficult due to complications (Roghanizad *et al.*, 2019; Yousefi *et al.*, 2021). The residual roughness in the root canal along with bacteria and their products can cause problems, although the use of washing solutions can play an effective role, it is difficult to completely clean these areas (Rodig *et al.*, 2019). Bacteria play an important role in root canal treatment failure, and one of the most important bacteria is *E. faecalis* (Gulabivala *et al.*, 2005). Biofilms protect bacteria, enabling growth and potentially causing dental issues like tooth decay and gum inflammation. Dental plaque is a common example of biofilm, consisting of various microorganisms, including fungi and bacteria. Both gram-positive (e.g., *E. faecalis)* and gram-negative (e.g., *Escherichia coli)* bacteria are known to form biofilms (Beigoli *et al.*, 2023).

Among the factors that cause bacterial resistance to root canal infection treatments are hyaluronidase, gelatinase, toxin, adhesion to surfaces, and extracellular superoxide production (Siqueira, 2001; Prada *et al.*, 2019). It is not possible to remove bacteria from inaccessible places and dentin tubules alone through mechanical and chemical preparation using tools and cleaning solutions (Kishen *et al.*, 2008). The presence of bacteria in the root canal reduces the long-term effectiveness of the treatment (Kayaoglu and Orstavik, 2008). The presence of persistent periapical lesions and the presence of *E. faecalis* have been reported in a comprehensive analysis of failed treatments (Ferreira *et al.*, 2015). In root canal treatments, the presence of this microorganism reduces the success of the treatment. For this reason, it is recommended to use drugs inside the canal to eliminate *E. faecalis* (Marickar *et al.*, 2015). Due to the widespread use of antibiotics when dealing with infections in the root canal of the tooth, as well as the emergence of bacteria that are resistant to these antibiotics, there are cases where this method of treatment is ineffective. As a result, alternative treatments with higher success rates become necessary. With the expansion of nanoscience and the use of nanoparticles with antibacterial properties, such as manganese oxide nanoparticles, attention has increased to their use in the treatment of resistant infections. Nanotechnology in medicine is important for both therapeutic applications and diagnostics. The use of nanocomposites in medical and scientific fields has increased significantly. Their small size creates a highly reactive surface, giving them unique chemical, physical, and biological properties, and they can easily overcome biological barriers in the body (Sabouri *et al.*, 2023; Narm *et al.*, 2024).

Calcium hydroxide is used as a common compound due to its appropriate biological effects inside the root canal of the tooth (Afkhami *et al.*, 2015). This compound has antimicrobial effects due to the release of hydroxyl ions and the creation of an alkaline environment (Dianat *et al.*, 2015). Hydroxyl ions cause inhibition in replication through the gap in the DNA molecule. It also causes disturbance in the enzymatic and metabolic activity of the cell and destroys the cytoplasmic membrane of microbes (Aguiar *et al.*, 2015).

Composites are composed of two main components, i.e. matrix and reinforcing agent, which have distinct physical and chemical properties. The matrix as a continuous phase can be composed of polymers, metals, or ceramics. Polymers show insufficient hardness and strength, metals have medium hardness and strength and at the same time high flexibility, while ceramics have high hardness and strength. The dispersed or discontinuous phase acts as a reinforcing agent and performs various functions such as increasing the physical and mechanical properties of the matrix (Sabouri *et al.*, 2023).

Composites have various applications in improving structural strength, electrical conductivity, biological compatibility, thermal properties, and environmental performance. They are classified into three main types: (1) multilayer composites in which the materials are bonded together by a matrix adhesive, (2) fibrous composites consisting of reinforcing fibers embedded in a matrix, and (3) particulate composites consisting of particles dispersed in a matrix (Sharma *et al.*, 2020). Manganese oxide is one of the nanoparticles with antibacterial properties. The use of this nanoparticle with calcium hydroxide and the formation of calcium hydroxide/manganese oxide nanocomposite can have an effective antibacterial effect in the treatment of tooth root canal infections, which is discussed in this research. The novelty of this research lies in using the Taguchi method to optimize the synthesis conditions of the nanocomposite. The purpose of this work is to investigate the antibacterial properties of a nanocomposite consisting of calcium hydroxide and manganese oxide against the oral pathogen *E. faecalis*.

2. MATERIALS AND METHODS

2.1. Synthesis of Manganese oxide nanoparticles

The production of manganese oxide nanoparticles in this study was done using the bacterial synthesis method. For this purpose, the bacterial strain (11083 IBRC-M) *Bacillus sp* obtained from the National Center of Genetic and Biological Resources of Iran was used. These bacteria can produce enzymes and metabolites that reduce and convert manganese ions into manganese oxide nanoparticles. In other words, bacteria metabolites act as a reducing agent and facilitate the biosynthesis process. After preparing the bacterial mass, in the next step, the centrifugation process was performed at a speed of 5000 rpm for 15 minutes to separate the supernatant. Then, 50 mL of solution containing 1 mg/mL of manganese acetate was added to a 250 mL Erlenmeyer flask containing 50 mL of supernatant solution. The solution was incubated in a shaker incubator, specifically with a rotation speed of 160 rpm, for 72 hours at 30 °C. Subsequently, the

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resulting solution, which contained nanoparticles, was subjected to filtration through filter paper to remove impurities. Finally, sterilization was done using a microbial filter (Safaei and Moghadam, 2022).

2.2. Synthesis of Manganese Oxide/Calcium Hydroxide nanocomposites

In this study, calcium hydroxide nanoparticles were obtained from Merck. To make calcium hydroxide/ manganese oxide nanocomposite and achieve the most favorable conditions to inhibit the growth of *E. faecalis* bacteria, the Taguchi test design method was used. This optimization method is considered more favorable compared to the usual methods (Taran *et al.*, 2017). In this method, 9 experiments were designed and three factors of calcium hydroxide (mg/mL) 150, 100, and 200, manganese oxide (mg/mL) 3, 6, and 9, and stirring time 60, 90, and 120 minutes were considered according to Table 1. In all 9 experiments, nanocomposite was prepared by the *in situ* synthesis method. In this method, different concentrations of calcium hydroxide and manganese oxide nanoparticles were prepared and sonicated for 10 minutes. Then, solutions containing calcium hydroxide were placed on a magnetic stirrer, and manganese oxide nanoparticles were added drop by drop. To form the nanocomposite, the solutions were placed on a magnetic stirrer at a temperature of 40 °C for 60, 90, and 120 min. To form the nanocomposite powder, the solutions were placed in an oven at a temperature of 80 °C (Safaei *et al.*, 2019).

2.3. Antibacterial activity

In this study, the antibacterial properties of nanocomposite consisting of calcium hydroxide and manganese oxide were investigated on *E. faecalis* (IBRC-M10740). This bacterium was obtained from the Center of Genetic and Biological Resources of Iran. After a 24-hour incubation period on the BHI culture medium, a suspension with a concentration equal to half McFarland was prepared from the mentioned bacteria. To investigate the antibacterial properties of calcium hydroxide/manganese oxide nanocomposite, the colony forming unit (CFU) method was used. Calcium hydroxide/manganese oxide nanocomposite was synthesized according to nine experiments designed by the Taguchi method, in which different concentrations of components were used. Then, for each test, 1 mL of the culture medium and 100 µl of bacteria were added to the nanocomposite. Next, after an 18-hour incubation period for all 9 experiments, 1 mL of the resulting mixture was added to the BHI medium using the pour-plate method. After incubation for 24 hours, the number of colonies was determined. This process was repeated for each nanocomposite and allowed a comprehensive assessment of the number of bacteria (Wang *et al.*, 2020).

2.4. Characterization

The components of this nanocomposite were determined using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), X-ray energy diffraction spectroscopy (EDX), element distribution map (Map) was investigated with SAMX detector, transmission electron microscope (TEM) and ultraviolet-visible spectroscopy (UV-Vis). These investigations were conducted to investigate the morphology and structural properties of calcium hydroxide, manganese oxide, and calcium hydroxide/manganese oxide nanocomposite samples. To perform the tests, Thermo infrared Fourier transform spectrometer, Philips X 'Pert X-ray diffraction device, TESCAN field emission scanning electron microscope MIRA III model, X-ray energy diffraction spectroscopy with MIRA III detector and element scattering map on the surface with the detector SAMX from TESCAN company MIRA II, TEM Philips EM208S transmission electron microscope and Shimadzu UV-160 A visible-ultraviolet spectrometer were used.

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity

To determine the most favorable conditions for the production of nanocomposite consisting of calcium hydroxide and manganese oxide with the strongest antibacterial properties, a total of 9 experiments were designed and carried out using the Taguchi method. After that, the effect of these synthesized nanocomposites on the survival of *Enterococcus faecalis* under different conditions was evaluated, as shown in Fig. 1. According to the data presented in Fig. 1, the antibacterial activity of nine synthesized nanocomposites was observed. Meanwhile, it was found that the nanocomposite related to test number 6 shows the highest level of antibacterial activity. Specifically, this nanocomposite included calcium hydroxide at a concentration of 150 (mg/mL), manganese oxide at a concentration of 9 (mg/mL) and a mixing time of 60 minutes. In this condition, the synthesized nanocomposite showed the strongest inhibitory effect on the growth of *Enterococcus faecalis* bacteria. The exact mechanisms by which nanoparticles exhibit antibacterial properties are currently under extensive investigation. Several mechanisms have been widely investigated, such as the induction of oxidative stress, the release of metal ions, and the generation of non-oxidative damage, all of which have been shown to affect the constituents of various microorganisms. Reactive oxygen species are a set of molecules or reactive intermediates that have significant oxidative potential despite their short existence in nature, and these species can potentially cause toxicity in microorganisms. Nanoscale materials are attached to the microorganism cell surface through active parts and then spread inside the cell and interact with different cell components.

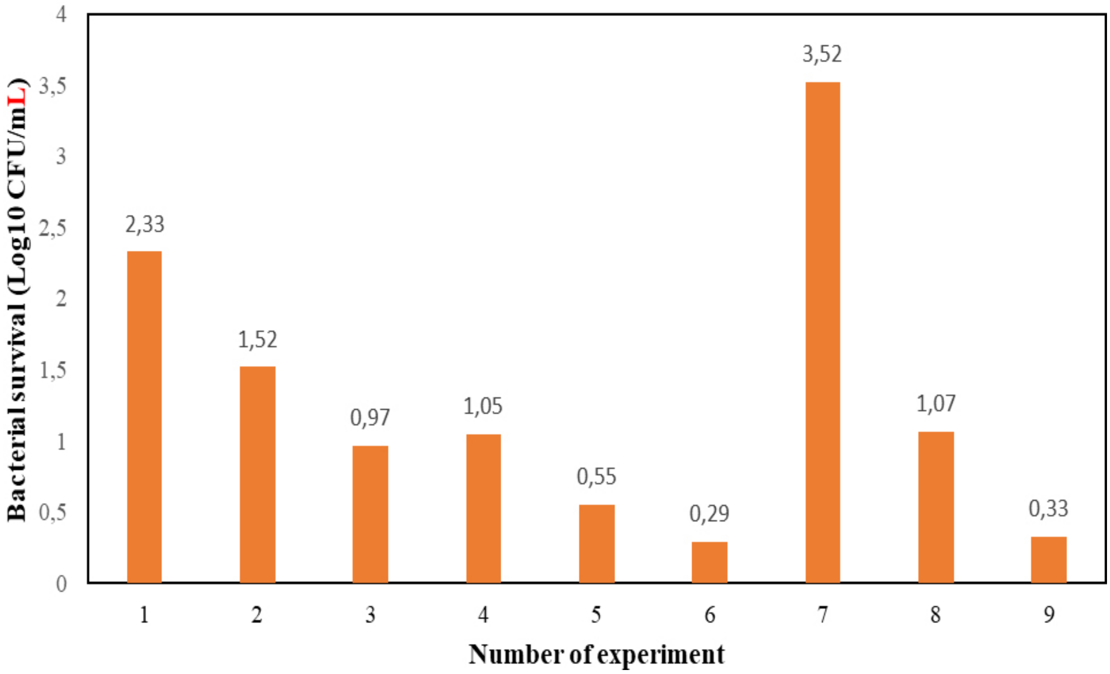

Figure 1. Taguchi design of experiments and effects of $Ca(OH)_2$ / MnO_2 synthesized nanocomposites on the survival rate of *E. faecalis.*

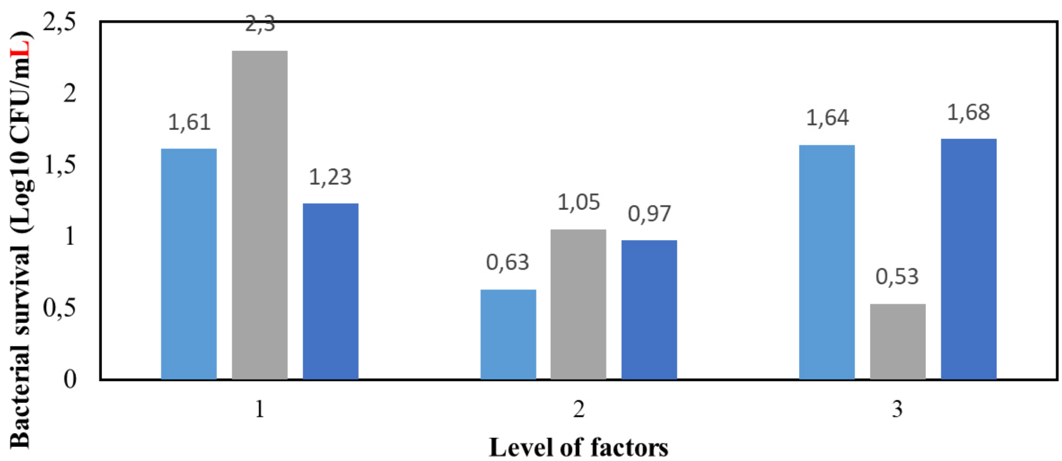

Figure 2. The main effects of different levels of $Ca(OH)_2$, MnO_2 and Stirring time on the survival rate of *E. faecalis.*

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In other words, by attaching to the cell wall of the microbe, these substances penetrate the cell membrane and cause destruction and disruption of the membrane (proteins, enzymes, and DNA). Nanoparticles produce ROS inside the cell, and these ROS induce oxidative stress, resulting in disturbed metabolic pathways and cell death (Safaei and Taran, 2022; Sarabikia *et al.*, 2023).

The antibacterial activity mechanism of calcium hydroxide and manganese oxide nanoparticles can be attributed to several factors. Manganese oxide nanoparticles can generate reactive oxygen species (ROS), which induces oxidative stress in bacterial cells, leading to cell damage and death. The dissolution of calcium hydroxide into calcium (Ca^{2+}) and hydroxyl (OH^{-}) ions creates a highly alkaline environment. This inhibits enzymatic activities at the bacterial cell membrane, disrupting metabolism, growth, and proliferation. The nanoparticles can attach to bacterial cell membranes, causing physical disruption and increased permeability, ultimately leading to cell lysis. These mechanisms collectively contribute to the antibacterial efficacy of calcium hydroxide and manganese oxide nanoparticles and their nanocomposite (Stankic *et al.*, 2016).

Fig. 2 shows the effect of calcium hydroxide, manganese oxide, and mixing time on the survival rate of *E. faecalis* bacteria. The findings indicate that the calcium hydroxide factor in the second level, the manganese oxide factor in the third level, and the mixing time in the second level have the greatest impact on the survival rate of *E. faecalis* bacteria.

The effect of different factors on the survival percentage of *E. faecalis* is shown in the diagram in Fig. 3. Among these factors, the second level of manganese oxide and the third level of mixing time showed a significant mutual effect on each other and also on the survival rate of *E. faecalis* with a value of 26.78.

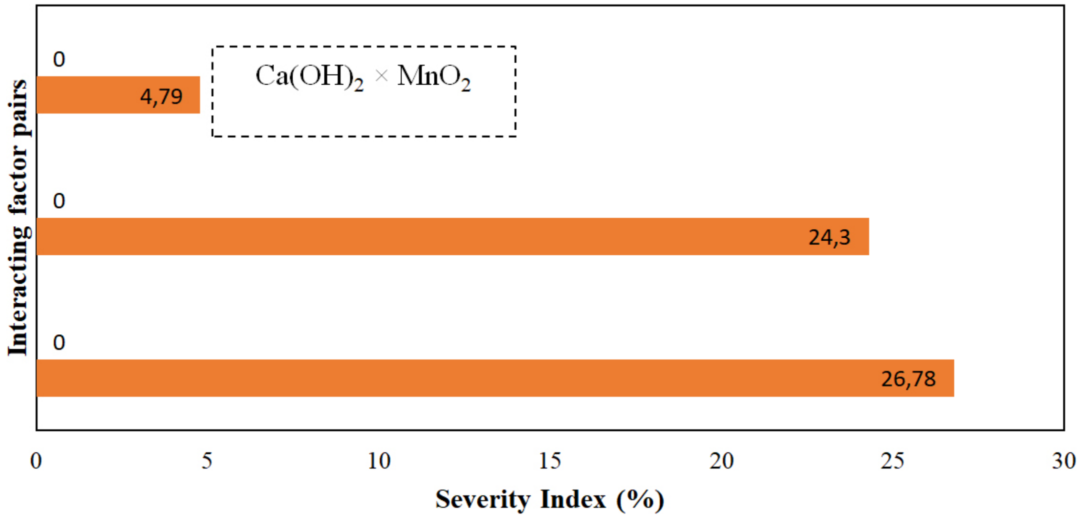

Figure 3. The interaction effects of studied factors on the survival rate of *E. faecalis*.

The interaction of calcium hydroxide in the first level and the duration of stirring in the third level showed an obvious reciprocal effect on the survival of *E. faecalis* bacteria, which was 24.30%. The minimum value of the mutual influence intensity index for calcium hydroxide in the first level and manganese oxide in the second level was observed as 4.79%.

Analysis of variance of the factors affecting the viability of *E. faecalis* is presented in Table 1. The greatest effect on the survival of *E. faecalis* was attributed to manganese oxide, whose effect was 44.53%, followed by calcium hydroxide with an effect of 10.43%. Conversely, the duration of mixing had no significant effect on the reduction of bacterial viability.

By analyzing the data and carefully examining the effect of each factor as well as their interaction, the optimal conditions required for the synthesis of calcium hydroxide/manganese oxide nanocomposite with the strongest antibacterial activity were estimated. This estimate is documented in Table 2.

Accordingly, manganese oxide showed the greatest effect on the survival rate of *E. faecalis*, while the least effect was related to the mixing time. On the other hand, calcium hydroxide produced an

Factors	DOF	Sum of Squares	Variance	F-Ratio (F)	Pure Sum	Percent (%)
$Ca(OH)_2$	2	1.98	0.99	1.87	0.92	10.43
MnO_2	2	4.97	2.49	4.70	3.91	44.53
Stirring time	2	0.78	0.39	0.74	0	0

Table 1. The analysis of variance of factors affecting the survival rate of *E. faecalis.* DOF, degree of freedom.

Factors	Level	Contribution
$Ca(OH)_2$	2	–0.66
MnO_2	3	–0.76
Stirring time	2	–0.33
Total contribution from all factors		–1.75
Current grand average of performance		1.29
Bacterial survival at optimum condition		–0.46

Table 2. The optimum conditions for the synthesis of $Ca(OH)_2$ / MnO_2 nanocomposites with the highest antibacterial activity.

effect that was between the above two factors and was close to manganese oxide. The most appropriate level for the factors of calcium hydroxide and mixing time was determined to be the second level, while the third level is the optimal choice for manganese oxide.

The findings of the research indicate that according to the data, the amount of bacterial colony growth in the presence of the synthesized nanocomposite under ideal conditions is determined to be –0.46. As a result, the nanocomposite consisting of calcium hydroxide and manganese oxide effectively prevents the activity of *E. faecalis* bacteria.

The results of the current study, along with previous articles, underscore the significant antibacterial activity of various nanocomposites, including calcium hydroxide/manganese oxide and other combinations. The study highlights the use of the direct mixing method for synthesizing the nanocomposite, while prior articles discuss alternative synthesis methods such as the sol-gel method and green synthesis. The potential application of the calcium hydroxide/manganese oxide nanocomposite in root canal treatment parallels other applications mentioned in the literature, including medical devices, food packaging, and wound healing. In evaluating antibacterial activity, the current study employs the Taguchi method. In contrast, previous articles refer to various characterization techniques and experimental setups for assessing the properties and efficacy of nanocomposites. These observations indicate that the current study's findings align with the themes and conclusions discussed in the prior literature.

3.2. FTIR analysis

The FTIR spectrum of the nanocomposite composed of calcium hydroxide and manganese oxide, as well as its components, is presented in Fig. 4 in the wavelength range of 400-4000 cm^{-1}. Graph a shows the FTIR spectrum of calcium hydroxide, where a sharp and prominent peak was detected in the region of 3640 cm^{-1}, attributed to the strain shown by the surface hydroxyl group. The observed peak in the absorption region of 1430 cm^{-1} is related to the presence of the carbonate anion group, especially the CO_3^{2-} group attached to calcium carbonate. The observed peaks in the region of 874 cm^{-1} were the result of the out-of-plane bending of the CO_3^{2-} type relative to the calcium hydroxide network. The observed peak in the region of 1640 cm^{-1} is the result of O-H bond vibration, which indicates the absorption of moisture and the presence of a water molecule inside these nanoparticles (Karthik *et al.,* 2017).

FTIR analysis of manganese oxide nanoparticles (diagram b) has shown the involvement of bacteria in the process of reducing and creating manganese oxide nanoparticles. The significant absorption detected in FTIR wavelengths, especially in the range of 3700 to 3200 cm^{-1}, indicates a strong interaction between water molecules (H-O-H) and the absorption of hydroxyl groups. The peak at 1616 cm^{-1} shows the bending impact of absorbed water. Mineral structures such as MnO have stronger bonds and weaker vibrations, which decrease the peak intensity in FTIR spectra.

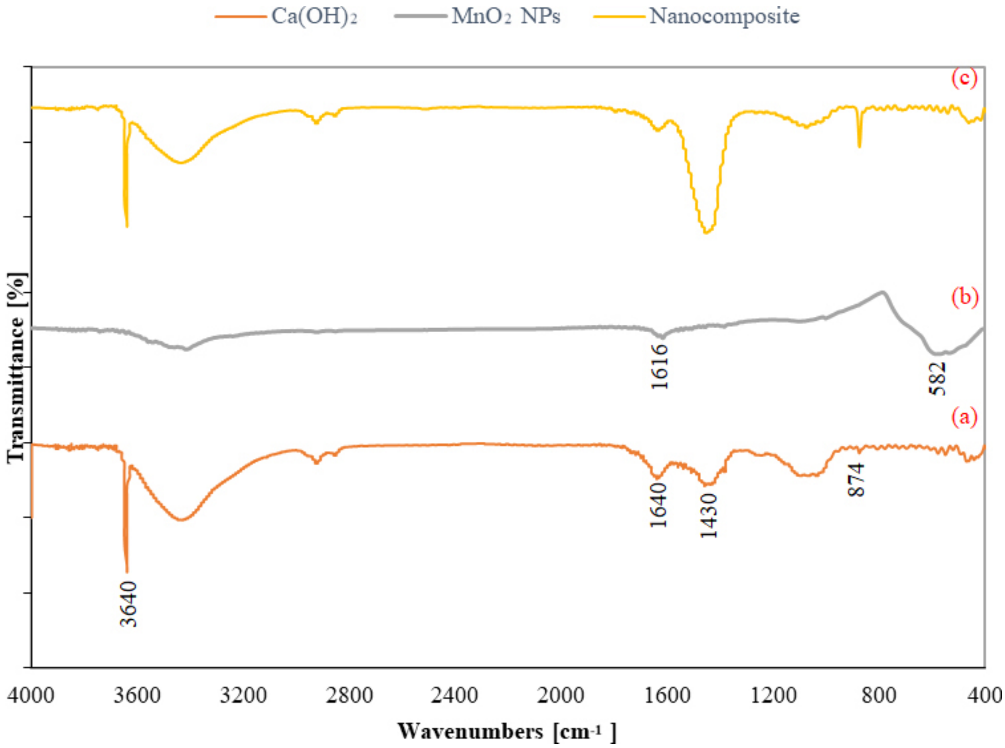

Figure 4. Infrared Fourier transform spectra of $Ca(OH)_2$ (a), MnO_2 (b), $Ca(OH)_2$ / MnO_2 nanocomposite (c).

The absorption peak between 400 and 800 cm^{-1} indicates O-Mn-O stretching collision. The FTIR spectrum showed distinct peaks at 582 cm^{-1}, which confirms the formation of manganese oxide nanoparticles MnO_2 (15). Comparing the FTIR spectrum of calcium hydroxide and manganese oxide nanoparticles and the spectrum of the manufactured nanocomposite showed that the spectrum of the nanocomposite is the result of the interaction between its components. In addition, the peaks due to the bonds within the components are also observed in the nanocomposite spectrum.

3.3. XRD analysis

Investigation of phase formation and crystal structure of nanocomposite and its components was done using X-ray diffraction (Fig. 5). The X-ray diffraction pattern of calcium hydroxide nanoparticles, (shown in graph a), showed the presence of a calcite phase characterized by a hexagonal crystal structure. Miller indices (hkl) were, (100), (101), (102), (110), and (111) corresponding to the angles 2θ, 28.62, 34.02, 47.17, 50.82, 54.37 (Safaei and Moghadam, 2022).

The diffraction pattern produced by X-ray spectroscopy of Manganese oxide nanoparticles, as shown in diagram b, has successfully proved the existence of the γ phase with the hexagonal structure of MnO_2. According to the separated peaks in the diffraction pattern, angles have been observed that the 2θ angles are equal 22.45 corresponding to the (120) plane, 37.15 corresponding to the (131) plane, 42.60 corresponding to the (300), 56.10 related to page (160) and 67.30 were related to plane (421) (Phuakkhaw *et al.*, 2016; Luo *et al.*, 2016).

The X-ray diffraction pattern of the calcium hydroxide/MnO_2 nanocomposite was shown (shown in graph c). This pattern showed a decrease in intensity as well as the removal or addition of characteristic peaks of the components. In addition, it was observed that these peaks in the X-ray diffraction spectrum of the synthesized nanocomposite are shifted to the left or right side compared to the diffraction pattern of the components. The observed phenomenon may be attributed to the changes in the distances between the crystal plates in the composition of the constituent components of the nanocomposite and thus indicate the formation of the final nanocomposite.

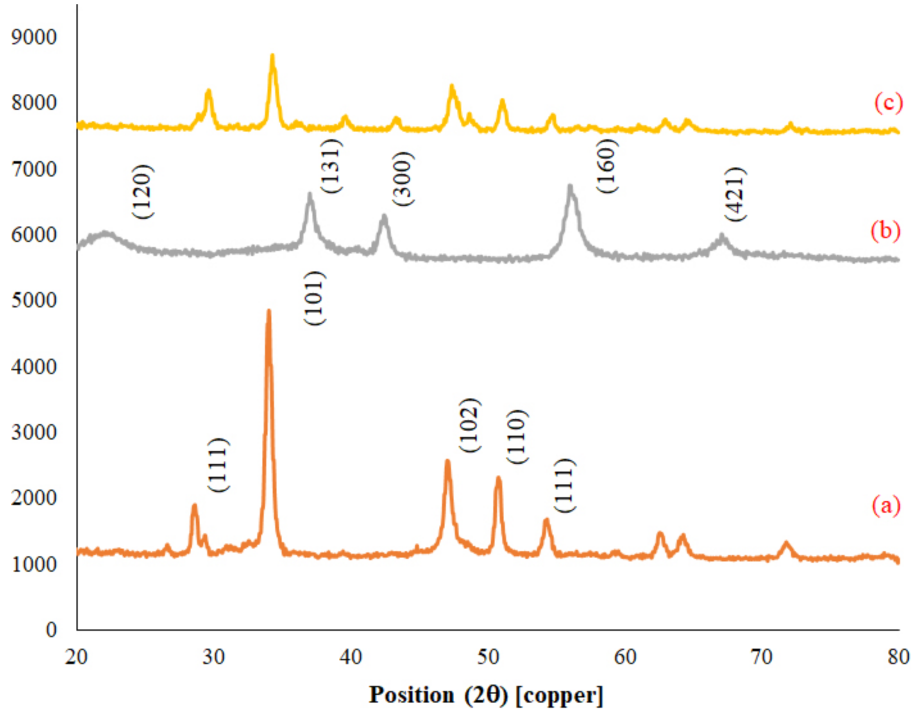

Figure 5. XRD Pattern of $Ca(OH)_2$ (a), MnO_2 (b), $Ca(OH)_2$ / MnO_2 nanocomposite (c).

3.4. SEM analysis

In Figure 6, the appearance and morphology of the nanocomposite consisting of calcium hydroxide and manganese oxide have been examined using a field emission scanning electron microscope. The image taken shows the presence of manganese oxide nanoparticles on calcium hydroxide and confirms the formation of nanocomposite.

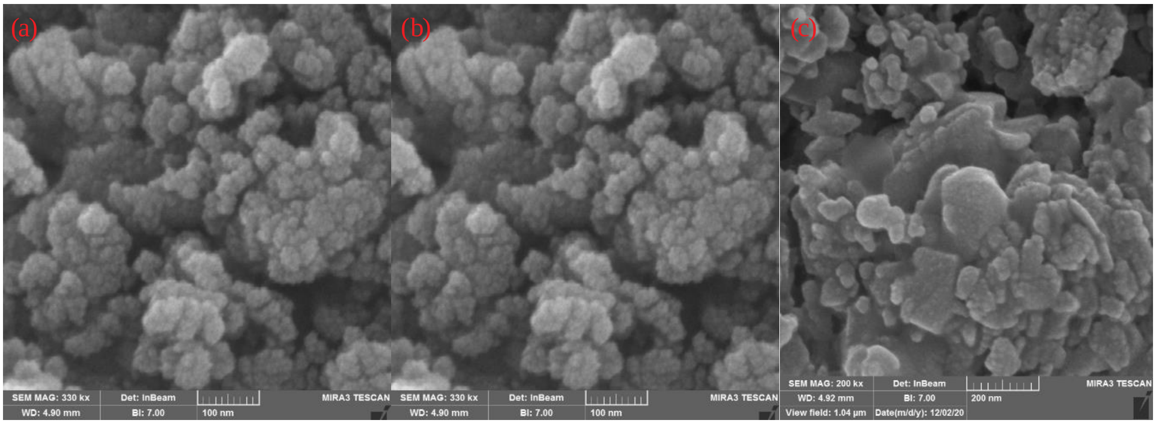

Figure 6. The scanning electron microscope image of $Ca(OH)_2$ (a), MnO_2 (b), $Ca(OH)_2$ / MnO_2 nanocomposite (c).

3.5. EDX analysis

Identification of the elements in calcium hydroxide samples, manganese oxide nanoparticles, and calcium hydroxide/manganese oxide nanocomposite was done using X-ray energy diffraction spectroscopy (Fig. 7) and made it possible to determine the constituent elements of these compounds. In the calcium hydroxide sample (diagram a), calcium elements with the highest peak intensity and highest weight percentage (58.79%) and oxygen elements (41.21%) were detected.

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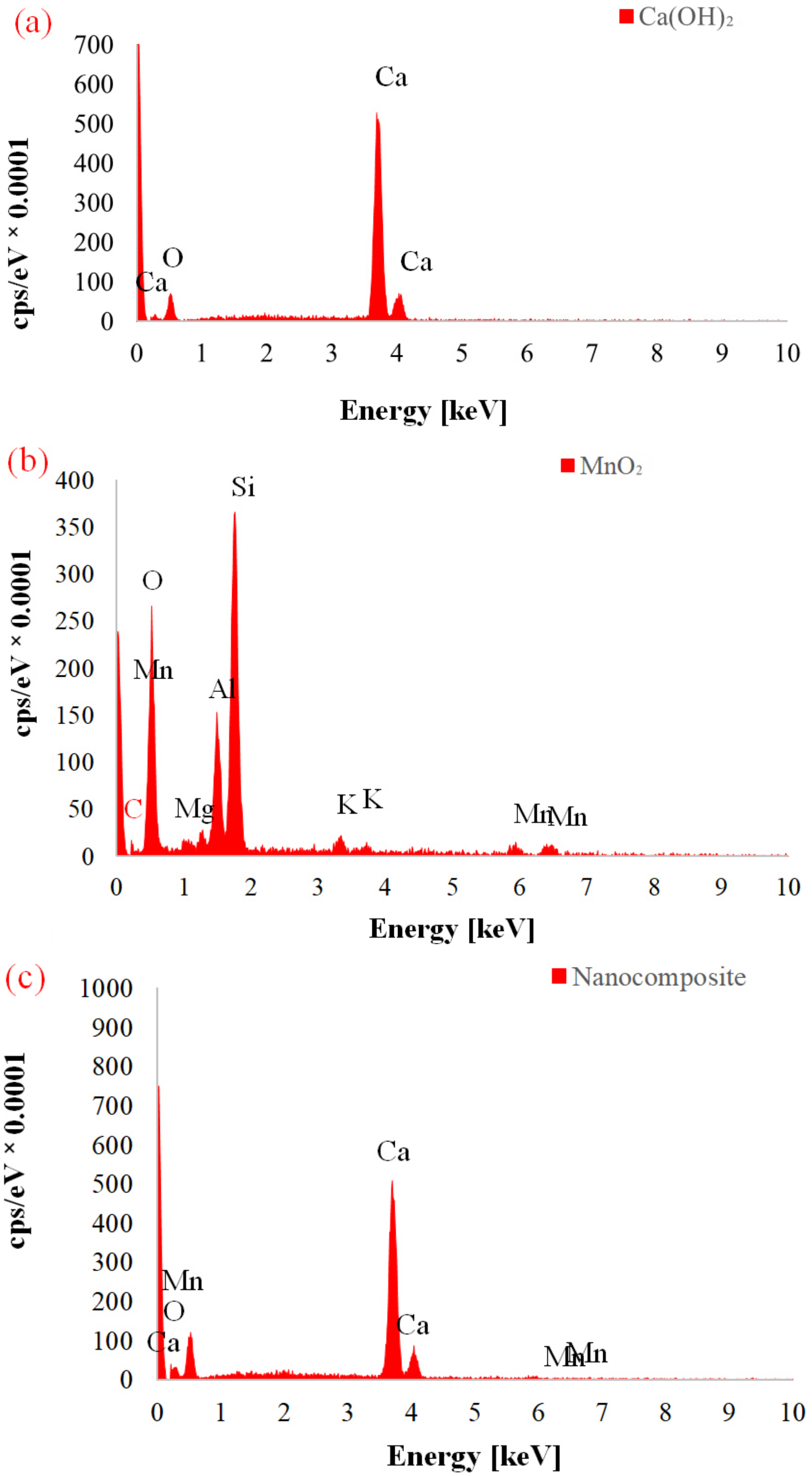

Figure 7. The energy dispersive X-ray (EDX) pattern of $Ca(OH)_2$ (a), MnO_2 (b), $Ca(OH)_2$ / MnO_2 nanocomposite (c).

EDX pattern of manganese oxide nanoparticles (Fig. 7b), the presence of carbon elements (5.96% by weight), oxygen (59.89% by weight), magnesium (1.04% by weight), aluminum (8.58% by weight), silicon (24.55% by weight), potassium (1.27% by weight) and manganese (1.70% by weight) and confirmed the nature of these nanoparticles. EDS analysis was performed on the synthesized nanocomposite, and the results indicated the presence of constituent elements in the final composition (Fig. 7). Among these elements, oxygen constitutes 90.47 percent by weight, while calcium and manganese constitute 51.28 percent and 0.81 percent by weight, respectively.

3.6. X-ray map analysis

The distribution map of calcium hydroxide/manganese oxide nanocomposite elements is shown in Fig. 8. This map shows the presence of calcium, manganese, and oxygen elements along with their distribution in the entire synthesized nanocomposite. The results of this test showed the uniform distribution of the constituent elements throughout the final structure of the nanocomposite and confirmed its creation.

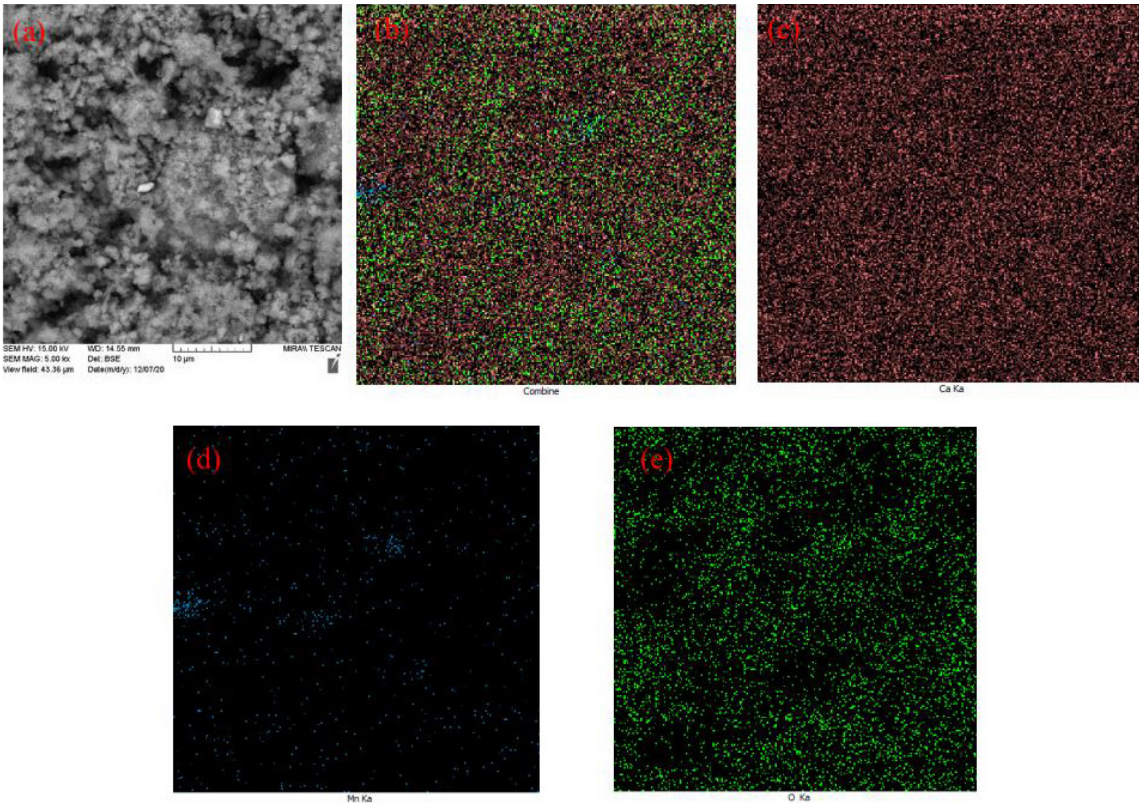

Figure 8. Dispersion map of composition components on the surface of final nanocomposite (a), all elements (b), Calcium (c), Manganese (d), Oxygen.

3.7. TEM analysis

The morphology of the nanocomposite consisting of calcium hydroxide and manganese oxide was investigated by preparing a TEM micrograph of the synthesized nanocomposite. Micrograph analysis showed that the Manganese oxide nanoparticles were effectively incorporated into the calcium hydroxide matrix, leading to the formation of this unique nanocomposite (Fig. 9).

3.8. UV-vis analysis

Visible and ultraviolet spectroscopic techniques were used to investigate the optical properties of nanocomposite including calcium hydroxide and manganese oxide as well as its components in the wavelength range of 200 to 800 nm. The diagram of this test is shown in Fig. 10. An oscillatory absorption band was detected in the spectrum of calcium hydroxide (as shown in graph a) in the wavelength

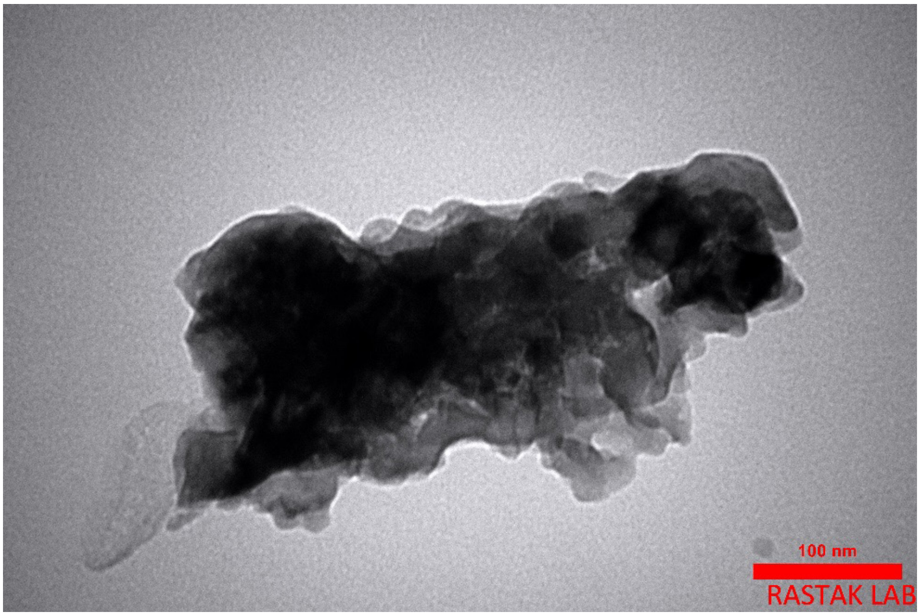

Figure 9. The transmitted electron microscope image of $Ca(OH)_2$ / MnO_2 nanocomposite.

range of 378 nm. This issue can be attributed to the different dimensions of calcium hydroxide particles in a certain range. In the spectrum associated with Manganese oxide nanoparticles (diagram b), two broad peaks at 275 and 363 nm showed absorption production for this particular sample. The reason for this was the existence of a range of different nanoparticle sizes. Finally, the absorption spectrum related to the fabricated nanocomposite (shown in diagram c) did not show a specific peak, and the main reason for that was the production of absorption in different and wide sizes. This can be attributed to the very high dispersion of nanocomposite particle size (Dianat *et al.*, 2015).

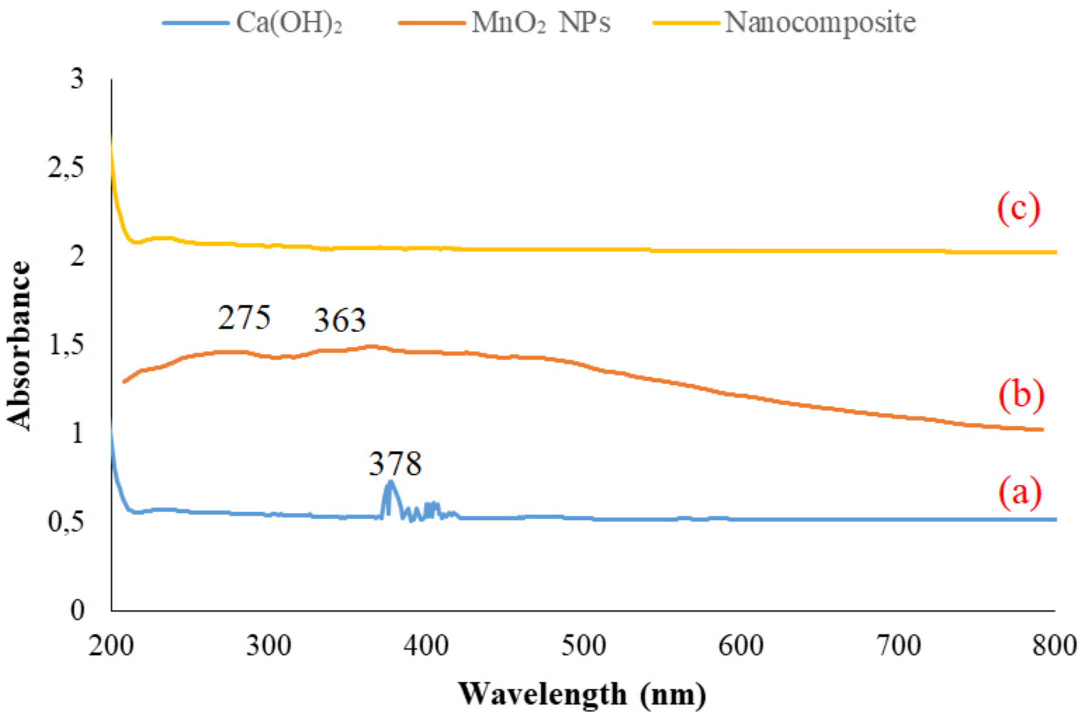

Figure 10. UV-Visible of $Ca(OH)_2$ (a), MnO_2 (b), $Ca(OH)_2$ / MnO_2 nanocomposite (c).

4. CONCLUSIONS

The antibacterial effect of calcium hydroxide/manganese oxide nanocomposite, which was synthesized by direct mixing method, was evaluated by examining the results of the experiments conducted using the Taguchi method. In particular, the evaluation focused on the activity of this nanocomposite against *Enterococcus faecalis* bacteria. According to the obtained results, it was estimated that the nanocomposite synthesized in optimal conditions (150 mg/mL of calcium hydroxide, 9 mg/mL of manganese oxide, and 90 minutes of stirring time) will completely prevent the growth of bacteria. The potential application of this nanocomposite in dealing with bacterial biofilms in root canals is predicted due to its antibacterial properties. The nanocomposite produced in this research can be used in many combined applications, such as filler in root canal treatment.

Building on our findings, we recommend the following directions for future research:

- In Vivo Studies: Perform in vivo studies to evaluate the biocompatibility and effectiveness of the nanocomposite in real-world medical applications, such as root canal treatments and other dental procedures.
- Exploration of other microbial strains: Investigate the nanocomposite's antimicrobial activity against a wider range of microbial strains, including antibiotic-resistant bacteria, to assess its potential as a versatile antimicrobial agent.
- Combination with Other Materials: Exploration of the synergistic effects of combining the nanocomposite with other materials, such as polymers or other nanoparticles, to enhance its properties and broaden its applications.
- Long-term Stability and Safety: Assess the nanocomposite's long-term stability and safety under various environmental conditions to ensure its reliability and effectiveness over extended periods.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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